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ORIGINAL ARTICLE

Synthesis of gold nanoparticles using herbal *Acorus calamus* rhizome extract and coating on cotton fabric for antibacterial and UV blocking applications

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KEYWORDS

Acorus calamus;
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UV-blocking

Abstract Gold nanoparticles (AuNPs) have been synthesized by greener method using chloroauric acid as precursor and extract of *Acorus calamus* rhizome as reducing agent. Formation of AuNP was confirmed by the presence of Surface Plasmon Resonance (SPR) peak in UV–Visible spectral analysis. XRD and FT-IR spectral analyses were performed for characterization. SEM images show spherical morphology and HR-TEM images reveal nanosize of AuNPs. The AuNPs were then coated on cotton fabric by pad-dry-cure method and characterized by SEM with EDAX technique. The results reveal the deposition of AuNPs on the surface of cotton fabric. Uncoated cotton, neat extract coated cotton and extract containing AuNPs coated cotton fabrics were then tested for antibacterial activity against Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli*) bacterial strains by AATCC 100 test method. It showed that the extract containing AuNPs coated cotton fabric had higher antibacterial activity than other test samples against *E. coli*. UV-DRS analysis performed on extract containing AuNPs coated cotton fabric showed improved UV-blocking property than uncoated cotton fabric and neat extract coated cotton fabric.

1. Introduction

Metal nanoparticles (MNPs) are interesting field of active research, because of their unique feature such as catalytic, optical, magnetic, electrical properties (Bindhu and Umadevi, 2015; Tang et al., 2014; Pan et al., 2014; Chander et al., 2014) and its extensive application in diverse areas such as biomedical, energy, catalysis, etc. (Lewis and Pikramenou, 2014; Chander et al., 2014; Noel et al., 2014) as well as due to their size and shape (Zhou et al., 2012; Mohanpuria et al., 2008).

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MNPs have been prepared by various methods such as chemical reduction (Boomi and Prabu, 2013), sonochemical (Sakai et al., 2014), microwave (Hameed and Sherif, 2015), greener (Yellappa et al., 2015; Shankar et al., 2014; Mariselvam et al., 2014; Suvardhan et al., 2014; Khalil et al., 2013; Gopinath et al., 2013), etc. Among the methods, greener method of synthesis is a simple, rapid, environment-friendly, non-toxic, cost-effective and convenient substitute for large scale production of MNP (Ashokkumar et al., 2015; Nalawade et al., 2014; Naveen Prasad and Padmesh, 2014; Sun et al., 2014; Rimal Isaac et al., 2013; Mallikarjuna et al., 2012).

Greener synthesis processes are gaining much attention as a possible alternative for the development of MNPs, where natural plant materials are used without any chemical reducing reagent (Bindhu and Umadevi, 2014, 2015; Basavegowda et al., 2014; Xu et al., 2014; Ashokkumar et al., 2015). Extracts of *Hibiscus cannabinus*, *Boerhaavia diffusa, Millingtonia hortensis*, *Enteromorpha flexuosa* J. Agardh, *Punica Granatum, Salicornia brachiata* and *Jasminum sambac* have been reported for the greener synthesis of silver and gold nanoparticles (Bindhu et al., 2014; Vijay Kumar et al., 2014; Ganesan et al., 2014; Yousefzadi et al., 2014; Lokina et al., 2014; Ahmed et al., 2014; Yellappa et al., 2015). Antibacterial activities of MNPs synthesized by greener method have been reported against various pathogens (Ahmed et al., 2014; Tran et al., 2013).

Multi-functional textiles are significant in recent years owing to awareness of health and hygiene aspects. The demand for making surface modification of fabric with nanoparticles is increasing, particularly fabrics holding antibacterial and UV-protecting properties. For the coating of MNPs on textile surfaces, various methods such as sonochemical (Subhranshu et al., 2010), sol–gel (Mahltig et al., 2005) and pad-dry-cure (Zahran et al., 2014) have been adopted. Interestingly, nanoparticles loaded on textile fabrics for antibacterial activity and ultraviolet protection efficiency have been studied and reported by many investigators (Wang et al., 2014; Tang et al., 2014; Basavegowda et al., 2014; Sivakumar et al., 2013; Abdel-Mohsen et al., 2012). In particular, antibacterial activity of cotton fabrics loaded with MNPs was reported and demonstrated against pathogens such as *Escherichia coli* (Ullah et al., 2014) and *Staphylococcus aureus* (Nateghi and Shateri-Khalilabad, 2014; Shateri-Khalilabad et al., 2013). Apart from these, NPs treated cotton fabrics also exhibited the long-term antibacterial activity together with laundering durability (Zahran et al., 2014; Liu et al., 2014) and UV production (Zheng et al., 2013). This might be due to the binding nature of MNPs onto the surface of the textile fabrics by existence of co-ordination and electrostatic interaction forces between MNP and some coordinating groups such as amino groups (Budama et al., 2013; Chang et al., 2009).

Acorus calamus is a natural plant belongs to the order Acorales and family Acoraceae. The genus name is *Acorus* and its species is called *A. calamus* (Lansdown, 2014). This plant has a very long history of medicinal use in Chinese and Indian herbal traditions (Motley, 1994; Howes et al., 2003). Throughout the history of civilization, it was used by the early Greeks and Romans. Hippocrates (460–377 BC) used this plant for medicinal purposes (Mabberley, 1990). This plant was present in Indian markets nearly two thousand years ago (Lloyd, 1929) and it has been sold as a medicine in every Indian shops (Sylvan et al., 2011). It was used for ailments such as dyspepsia (Wren, 1956), mouth and throat diseases, fevers, epilepsy, bronchitis, hysteria, tumors, rat bites, ear worms, toothaches, pains of the chest and kidneys, insomnia, melancholia, neurosis, loss of memory depression and mental disorders (Kirtikar and Basu, 1975), asthma, diarrhea, dysentery, flatulence (Jain, 1968; Kim et al., 2009; Nandakumar et al., 2013).

In Arabic culture, the rhizome has been used to cure many diseases such as stomachic, diaphoretic, diuretic, vertigo, headaches (Barton and Castle, 1877). This plant was also mentioned in the Chester Beatty papyrus dating to approximately 1300 BC (Indigo.ie, 2007). The plant was introduced to Britain in the late 16th century, by at least 1596 true *A. calamus* was grown in Britain (Husken, 1996). Modern research on *A. calamus* shows neuroprotective effect against stroke and chemically induced neurodegeneration in rats (Rabadia et al., 2014). Also, roots of *A. calamus* have shown antioxidant (Barua et al., 2014), antimicrobial and insecticidal activities (Kumar et al., 2014).

The major active and distinct chemical components in *A. calamus* rhizomes are asarone (α- and β-), caryophyllene, isoasarone, methyl isoeugenol and safrole (Namba, 1993; Raina et al., 2003; Radusiene et al., 2007; Deepak and Ashwani, 2011; Prabodh et al., 2013; Ashwani et al., 2014; Asha Devi et al., 2014).

Synthetic methods of preparing MNPs using chemical reducing agent pose toxicity or health hazards. Therefore, development in the synthesis of AuNPs from natural plant extract is considered to be the most appropriate method on the environmental issues. It is observed that *A. calamus* has been used traditionally as herbal medicine for numerous pharmacological applications. No report is available on the antibacterial activity together with UV-protection using *A. calamus* rhizome. Hence, it was aimed to study the green synthesis of AuNPs using the extract of *A. calamus* rhizome for antibacterial and UV blocking applications.

2. Experimental

2.1. Materials and methods

A. calamus rhizomes were purchased from ayurvedic medical shop at Karaikudi town located in India. High pure water was obtained from TKA-LAB Reinst water system. $HAuCl_4 \cdot 3H_2O$ was purchased from Sigma Aldrich and used as such. Buffer pH tablets were procured from Ranbaxy and used as such. Cotton fabric was purchased from local pharmacy outlet. Bacterial strains of *S. aureus* (MTCC 96) and *E. coli* (MTCC 1671) were tested at Center for Marine pharmacology, School of Marine Sciences, Alagappa University, India.

2.2. Preparation of *A. calamus* rhizome extract

A. calamus rhizome was subjected to extraction at three different temperatures (room temperature, 60 °C and 100 °C); for the extraction at room temperature, 30 g of washed rhizome was neatly grounded with 90 ml water and kept for 15 min and filtered. For the extraction at 60 °C and at 100 °C, 30 g of washed rhizome was grounded with 90 ml water and heated for 15 min using soxhlet apparatus at 60 °C and at 100 °C separately and filtered. The filtrates thus obtained by the said methods were used as bio-reducing agent to synthesize AuNPs.

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2.3. Synthesis of AuNPs

In the Erlenmeyer flask, 2.5 ml of appropriate extract, 2.5 ml of 0.001 M chloroauric acid and 1 ml of appropriate pH (4, 7 and 9.2) buffer solution were added. This mixture was allowed to stir at 240 rpm using magnetic stirrer. The reduction of Au^{3+} to Au^{0} was monitored by observing change in color of the reaction mixture from light brown to dark brown. This procedure was extended with higher concentration of chloroauric acid (0.01 M) to understand the effect of concentration.

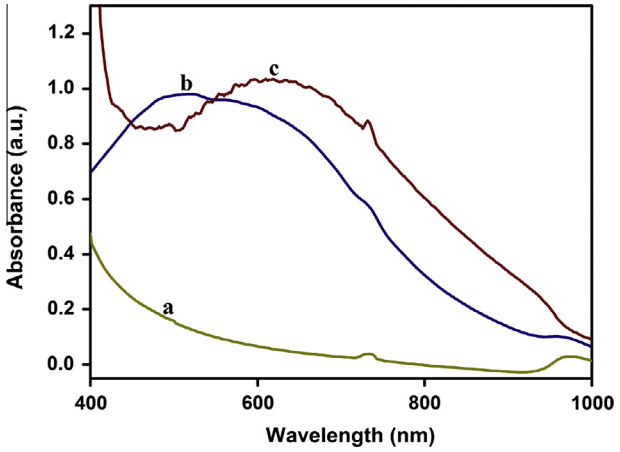

Figure 1 UV–Vis spectra of (a) pristine extract, (b) extract containing AuNPs (0.001 M) and (c) extract containing AuNPs (0.01 M).

2.4. Coating on cotton fabric by pad-dry-cure method

The pristine extract was coated on cotton fabric by pad-dry-cure method. The fabric was fed into the padding mangle containing 100 ml of *A. calamus* extract. Padding process was carried out for 15 min at ambient conditions. The coated fabric was taken out, washed with pure water and air dried. The coating procedure was extended for the extract containing AuNPs also.

2.5. Instrumentation

The synthesis of AuNPs was monitored using UV–Vis spectrophotometer (Jasco-V-530) by the formation of Surface Plasmon Resonance (SPR) peaks. The extract containing AuNP was dried and the powder was characterized using X'Pert PRO XRD instrument. FT-IR spectra of analytes were recorded using BRUKER Optik GmbH-TENSOR 27 instrument. Surface morphology of fabric and shape of AuNPs were studied using HITACHI S3000H SEM at 15 kV instrument. Particle size of AuNPs was obtained using high resolution transmission electron microscope (HR-TEM) JEOL-JEM 2100 operating at 200 kV. The UV-blocking property of the

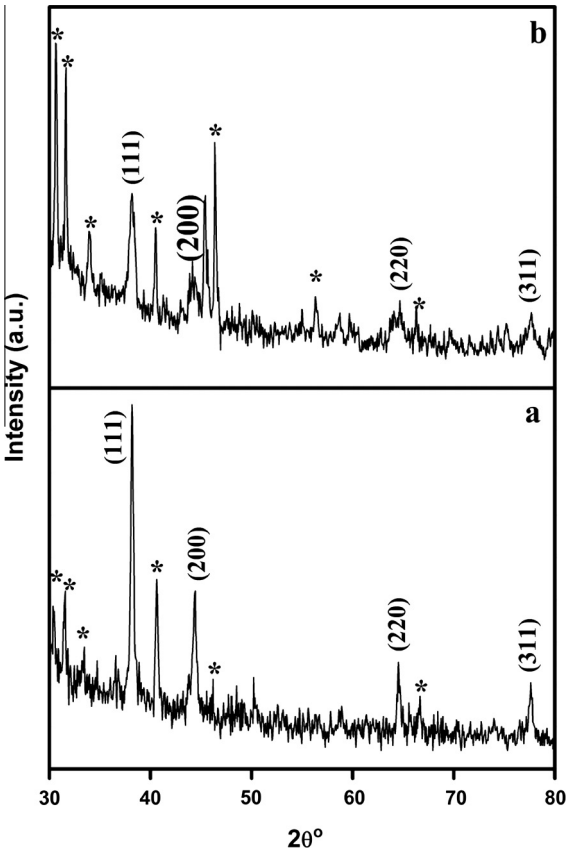

Figure 2 XRD pattern of synthesized AuNPs from (a) 0.001 M and (b) 0.01 M chloroauric acid.

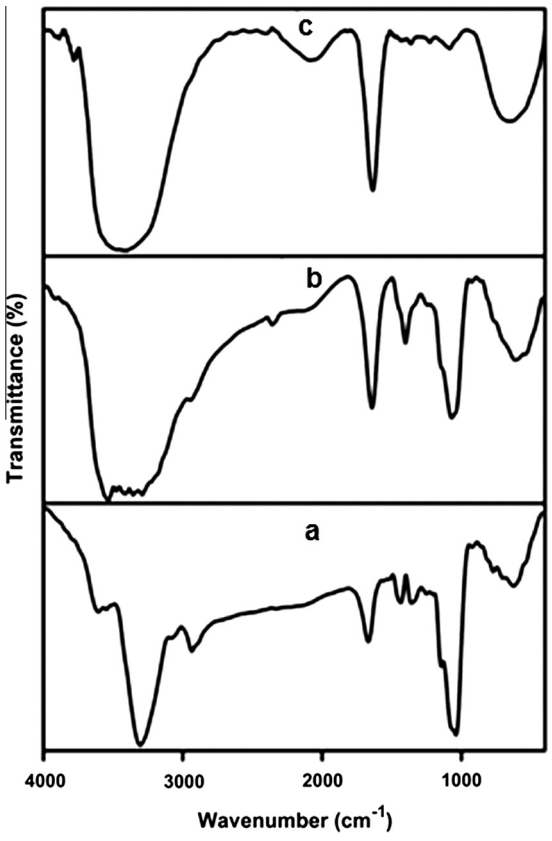

Figure 3 FT-IR spectra of (a) neat extract, (b) extract containing AuNPs (0.001 M) and (c) extract containing AuNPs (0.01 M).

samples was examined by UV-diffuse reflectance (UV-DRS) spectral analysis using Carry 5000 spectrophotometer as per Australian/New Zealand AS/NZS 4399:1996 standards.

2.6. Antibacterial activity test

The antibacterial activity of uncoated cotton, neat extract coated cotton and the extract containing AuNPs coated cotton fabrics was evaluated against *S. aureus* and *E. coli* by AATCC 100 test method. The percentage reduction was calculated using the equation $R(\%) = ((A - B)/B)100$, where R = % reduction, A is the number of bacteria recovered from the inoculated test swabs in the jar after incubation with raw sample, B is the number of bacteria according to "A" conditions with antibacterial modified cotton sample.

3. Results and discussion

In the extraction process at three different temperatures (room, 60 °C and 100 °C), at two different concentrations of precursor $HAuCl_4 \cdot 3H_2O$ (0.001 M and 0.01 M), and at three different pH conditions (4, 7 and 9.2), the results reveal that the SPR band of AuNPs was obtained for both 0.001 M and 0.01 M gold precursors at a pH of 7 with the extract obtained at room temperature. But, the extract obtained at either 60 or 100 °C and the pH condition either at 4 or at 9.2 did not show the characteristic SPR corresponding to AuNPs. Thus, the room temperature extraction and a pH of 7 were considered as optimum in the synthesis of AuNPs.

3.1. UV–Vis analysis

Fig. 1 shows UV–visible spectra of neat extract (curve a) and extract containing AuNPs obtained from 0.001 (curve b) and 0.01 M chloroauric acid (curve c). Bands are observed at 518 nm (0.001 M) and 622 nm (0.01 M), which can be attributed to the SPR of AuNPs (Kasthuri et al., 2009). It is noted that lower concentration (0.001 M) shows SPR band at shorter wavelength and higher concentration (0.01 M) shows band at longer wavelength. The observation of broad and shift in SPR band might depend on size, shape and aggregation in the formation of Au^0 from Au^{3+} (Chen and Goodman, 2004; Liang et al., 2007). Neat extract did not show characteristic SPR band. Thus, it can be concluded that the SPR band observed at 518 nm and 622 nm is due to the formation of AuNPs only (Kasthuri et al., 2009).

3.2. XRD analysis

AuNPs synthesized from precursors at different concentrations such as 0.001 M and 0.01 M are shown in Fig. 2. Peaks at 38°, 44°, 64° and 77° are assigned to the face centered cubic (fcc) units of Au, which are represented by Bragg diffraction planes of (1 1 1), (2 0 0), (2 2 0) and (3 1 1) respectively [JCPDS file No. 01-089-3697]. Apart from aforementioned peaks, additional peaks are also observed, which are indicated by asterisk. These may be due to the bio-inorganic compounds and protein matters present in the extract (Shankar et al., 2005). The aver-

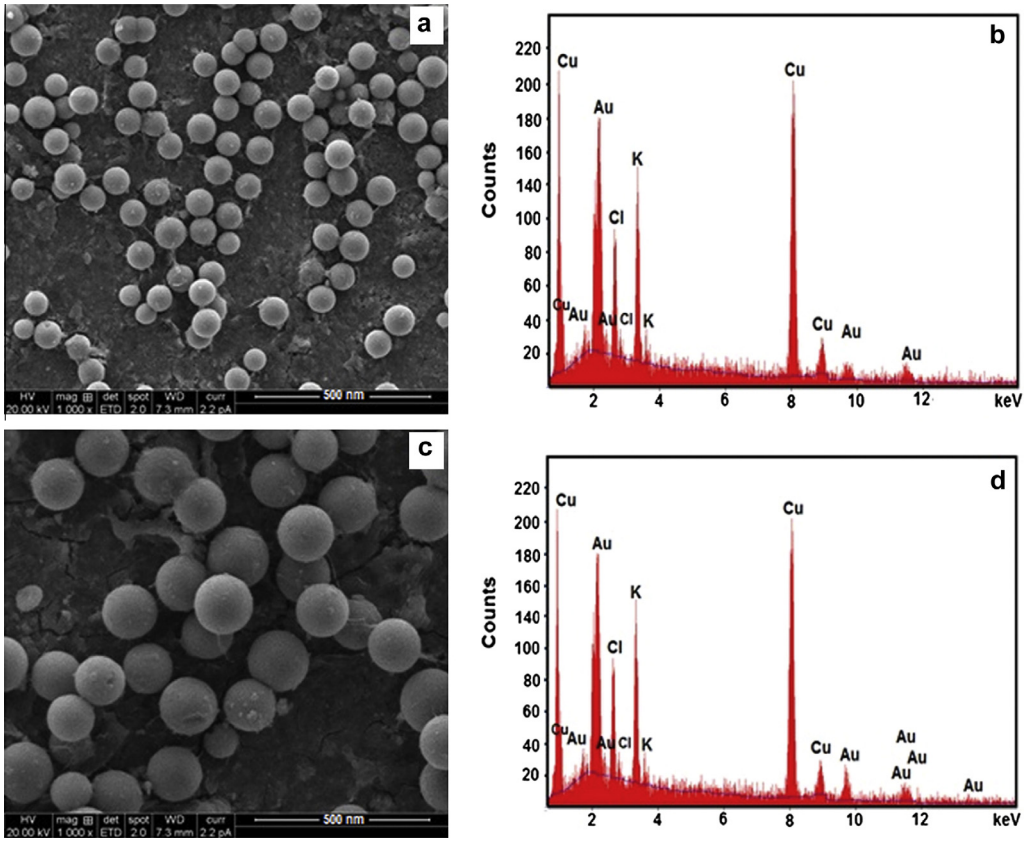

Figure 4 SEM with EDAX images of AuNPs obtained from (a and b) 0.001 M and (b and d) 0.01 M chloroauric acid.

age grain sizes calculated using Scherrer formula are 15 nm (with 0.001 M) and 20 nm (with 0.01 M) chloroauric acid.

3.3. FT-IR analysis

FT-IR spectra were recorded for neat extract and extract containing AuNPs (Fig. 3). They show peaks at 1669, 1445 and 1346 cm^{-1} (curve a). Peak observed at 1669 cm^{-1} can be assigned for amide I group from protein part of extract, which may be responsible for the reduction of Au^{3+} ions to Au^0. Peaks at 1445 and 1346 cm^{-1} are related to the C–H bending vibrations of the aromatic tertiary amine group. Peaks at 1640, 1462 and 1626 cm^{-1} are attributed to the N–H, C–H bending and secondary amine respectively (curve b). Peaks at 1626 and 1095 cm^{-1} are ascribed to N–H bending and C–N stretching respectively (curve c). From the FT-IR results, the peak for amine group (curves b and c) is observed to shift in peak position. This may be due to the involvement of the amide I group in the reduction of Au^{3+} to Au^0. It is reported that amine group is acting as a capping agent (Rimal Isaac et al., 2013; Khalil et al., 2012; Shankar et al., 2004).

3.4. SEM with EDAX analysis

Fig. 4 illustrates SEM micrograph with EDAX spectrum of AuNPs synthesized from two different concentrations (0.001 and 0.01 M chloroauric acid). AuNPs obtained from lower concentration (0.001 M) show uniform in size with smaller spherical ball morphology with higher distribution (image a). The particle size observed is below 100 nm. Synthesis from higher concentration (0.01 M) shows bigger spherical ball in shape with lower distribution (image c). The particle size observed is in the range between 100 and 500 nm. EDAX spectrum reveals the presence of Au^0 in the extract containing AuNPs (images b and d). These results reveal that the size of AuNPs has direct relation with the concentration level of the precursor.

3.5. HR-TEM analysis

HR-TEM with SAED images of AuNPs synthesized from different concentrations (0.001 M and 0.01 M) of chloroauric acid is shown in Fig. 5. Lower concentration has resulted in spherical shape Au^0 (image a) with higher distribution and the average particle size is noted as 10 nm. Higher concentration also resulted in spherical shape Au^0 (image b) with lower distribution and the average particle size is noted as 10 nm. SAED (images c and d) results show bright circular rings corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of Au^0.

From the SEM and HR-TEM results, it is observed that the AuNPs obtained from lower concentration (0.001 M) of chloroauric acid produced admirable results (morphology, size and distribution) than from higher concentration (0.01 M). Thus, further studies were carried out with samples used with 0.001 M chloroauric acid.

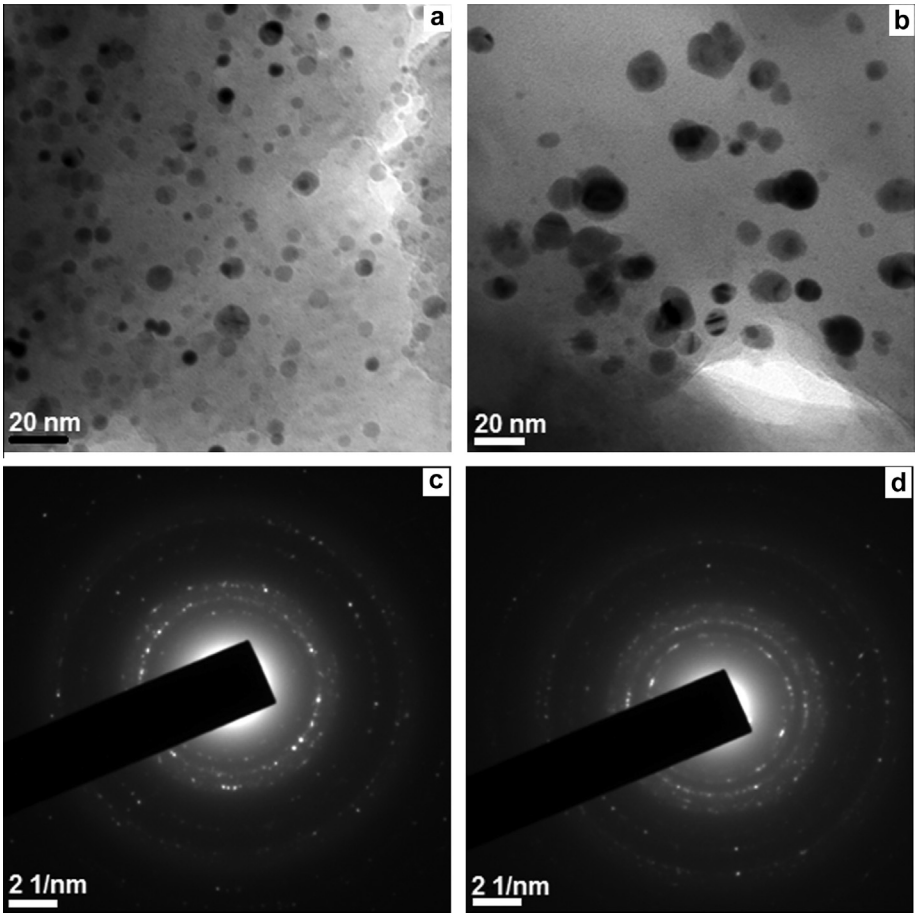

Figure 5 HR-TEM with SAED images of AuNPs obtained from (a and c) 0.001 M and (c and d) 0.01 M chloroauric acid.

3.6. SEM with EDAX of AuNPs coated on cotton fabric

Fig. 6. shows SEM with EDAX spectra of uncoated and AuNPs coated cotton fabrics. Clear existence of AuNPs (bright spots in image e) is observed on extract containing AuNPs coated cotton fabric when compared with uncoated cotton (image a) and pristine extract coated cotton fabric (image c). EDAX spectrum (image f) is also entrenched the presence of Au^{o} in extract containing AuNPs coated cotton fabric (Zahran et al., 2014; Nateghi and Shateri-Khalilabad, 2014).

3.7. Antibacterial activity

Antibacterial activity of uncoated cotton, neat extract coated cotton and extract containing AuNPs coated cotton was analyzed by quantitative test method against *S. aureus* and *E. coli* bacteria at different specified time durations of 24 and 48 h. The noticeable percentage inhibition is presented in Table 1. It reveals that the extract containing AuNPs coated cotton fabric shows better antibacterial activity than that of neat extract coated cotton and uncoated cotton. The percentage inhibition was found to increase significantly after 48 h compared to 24 h. This may be due to the presence of impregnated AuNPs on the surface of cotton fabric (cellulosic matrix) assisted by hydroxyl groups present in the *A. calamus* extract (Ullah et al., 2014; Zahran et al., 2014; Muthuswamy et al., 2010). The hydroxyl groups present in the extract might stabilize the AuNPs on the surface of cotton consisting repeating chain units of the 4-d-glucosepyranose texture, which might produce more surface area for more adsorption of AuNPs. The cotton immobilized with extract containing AuNPs without the use of any binder showed better antibacterial activity than uncoated cotton and neat extract coated cotton.

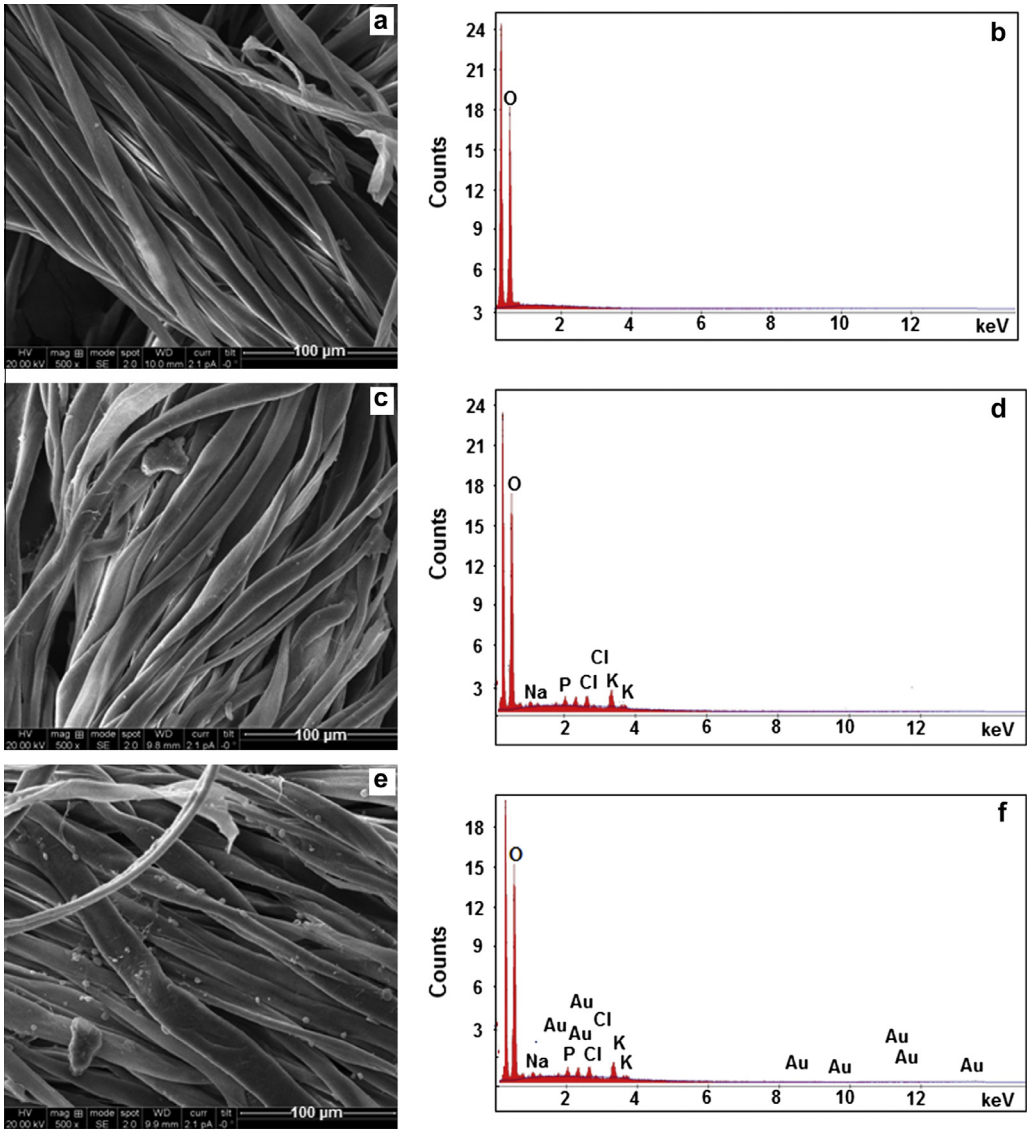

Figure 6 SEM with EDAX images of (a and b) uncoated cotton, (c and d) extract coated cotton and (e and f) extract containing AuNPs coated cotton.

Table 1 Antibacterial efficiency of AuNPs synthesized using *Acorus calamus* extract prepared at room temperature.

Nature of the sample	Percentage reduction			
	Escherichia coli		*Staphylococcus aureus*	
	24 h	48 h	24 h	48 h
Uncoated cotton	9.37	41.8	5.8	34.4
Neat extract coated cotton	53.1	55.2	41.1	43.9
Extract containing AuNPs coated cotton	58.0	80.3	50.5	63.9

It is reported that (Sivakumar et al., 2013) the antibacterial activity of AuNPs loaded cotton fabrics by pad-dry-cure method possessed some extent of washing stability. This stability might be due to the presence, coordination and electrostatic interaction of forces between AuNPs and amino groups present in the extract (Liu et al., 2014; Chang et al., 2009; Fouda).

3.8. UV-DRS spectral analysis

UV-blocking property (Fig. 7) of uncoated cotton (curve a), neat extract coated cotton (curve b) and extract containing AuNPs coated cotton (curve c) was studied. Ultraviolet protection factor (UPF) was computed using the equation $\mathrm{UPF} = \frac{\sum_{280\ \mathrm{nm}}^{400\ \mathrm{nm}} E_\lambda S_\lambda \Delta_\lambda}{\sum_{280\ \mathrm{nm}}^{400\ \mathrm{nm}} E_\lambda S_\lambda T_\lambda \Delta_\lambda}$, where S_λ is spectral irradiation of the skin in UV region (280–400 nm), E_λ is relative erythemal spectral effectiveness, T_λ is spectral transmittance of the fabric, Δ_λ is increment relating to wavelength and λ is wavelength in nanometer (Zheng et al., 2013). Extract containing AuNPs coated cotton fabric showed very good UV protection efficiency with an UPF value of 31.9. Uncoated cotton and neat extract coated cotton fabrics showed only marginal values of 3.9 and 4.3 respectively.

From the image (Fig. 6) of SEM coupled with EDAX, it is noted that AuNPs are deposited not only on the surface of the cotton, but also on the spaces between the yarns in cotton (Tang et al., 2014; Sivakumar et al., 2013; Hallaih et al., doi: 10.1177/1528083713485612; Vigneshwaran et al., 2006). Thus, the presence of extract containing AuNPs on cotton fabric might prevent the penetration of UV radiation through the fabric.

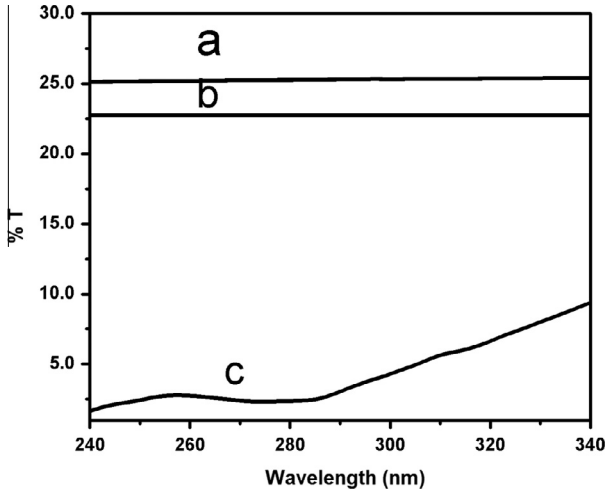

Figure 7 UV-DRS spectrum of (a) uncoated cotton, (b) neat extract coated cotton and (c) extract containing AuNPs coated cotton.

4. Conclusions

In this study, AuNPs have been synthesized from chloroauric acid at ambient condition using natural plant *A. calamus* extract as greener method. The formation and sizes of AuNPs were depending on precursor concentration. Spherical morphology of AuNPs was observed. *A. calamus* extract containing AuNPs coated cotton fabric showed improved antibacterial activity and UV-DRS efficiency than uncoated cotton and neat extract coated cotton. These results could be applied to medical textiles.

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